β-Sitosteryl D-Glucoside and β-Sitosterol from Commercially Dried Grapefruit Pulp[1]

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Introduction

Extracts of commercially dried grapefruit pulp[3] were tested in this laboratory for antibiotic activity. Methanol extracts exhibited some antifungal activity but little or no antibacterial activity. In isolating the material responsible for the antifungal activity, many of the compounds previously reported in grapefruit (1, 2) were obtained. Although neither β-sitosteryl D-glucoside nor β-sitosterol has been isolated previously from grapefruit, these two compounds accounted for most of the antifungal activity observed. β-Sitosterol was isolated from grapefruit seeds in larger amounts, but only a trace of β-sitosteryl D-glucoside was found in the seeds. Swift (3) has recently published a report on the isolation of β-sitosteryl D-glucoside from the juice of Florida Valencia oranges (*Citrus sinensis* L.) and has supplied us with samples for comparison.

Experimental

Isolation of β-Sitosteryl D-Glucoside

Approximately 150 kg. of commercially dried grapefruit pulp, consisting mainly of peel and seeds, was washed with hot water to remove naringin, sugars, salts, and other soluble substances, and the washings were discarded. Water removed about 40% of the total solids. The

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[2] One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, United States Department of Agriculture.

[3] Kindly supplied by Dr. E. M. Burdick, Texsun Citrus Exchange, Weslaco, Texas.

remaining solids were dried in a forced-draft electric oven and then extracted batchwise at room temperature with three portions of methanol. The methanol extract was concentrated at reduced pressure to an approximate concentration of 1.0 ml./g. of dried starting material. A precipitate that formed on cooling the methanol concentrate was collected by centrifugation. The precipitate, containing most of the β-sitosteryl D-glucoside, was washed once with peterolum ether to remove adhering oil. The methanol supernatant was admixed with 1 vol. each of water and petroleum ether. The methanol–water–petroleum mixture (1:1:1) separated into two layers with solid material at the interface, which was found to consist mainly of β-sitosteryl D-glucoside. The petroleum ether layer containing oils and the methanol–water layer containing large amounts of naringin and sugars were discarded. The solid from the interface was collected and combined with the precipitate from the methanol concentrate. The combined solids were treated with acetone to remove limonin and free β-sitosterol. The acetone-insoluble portion was treated with 0.1 *N* sodium hydroxide solution to remove hesperidin (0.08 % of washed dried pulp). The β-sitosteryl D-glucoside, which was insoluble in alkali, was then crystallized from hot 95 % ethanol. The yield of pure β-sitosteryl D-glucoside from 150 kg. of dried grapefruit pulp was 8.65 g. (0.0058 %). The β-sitosteryl D-glucoside melted at 290–291°C. in an evacuated sealed capillary tube.[4] It was insoluble in water, alkali, acids, and most organic solvents, but was sparingly soluble in methanol and ethanol, and very soluble in pyridine; $[\alpha]_D^{21}$ $-46.9 \pm 1.5°$ in freshly distilled pyridine, [lit., $[\alpha]_D^{20}$ $-40.1°$, pyridine (3)].

Anal. Calcd. for $C_{35}H_{60}O_6$: C, 72.87; H, 10.48. Found: C, 72.33; H, 10.37.

β-Sitosteryl D-Glucoside Tetraacetate

β-Sitosteryl D-glucoside (100 mg.) was dissolved in 10.0 ml. of dry pyridine in a glass-stoppered flask, and 2.0 ml. of acetic anhydride was added. After standing overnight at room temperature, the reaction mixture was transferred to a separatory funnel and diluted with 50 ml. of distilled water. A white precipitate formed, which was extracted with three 40-ml. portions of chloroform. The chloroform extract was filtered through a funnel provided with a cotton filter previously saturated with chloroform. The chloroform extract was concentrated to dryness; the

[4] All melting points reported in this paper are uncorrected. They were determined in capillary tubes immersed in an electrically heated oil bath.

product was dried in a vacuum oven and then recrystallized three times from hot 95% ethanol, m.p. 165–165.5°C.; $[\alpha]_D^{21}$ −36.8 ± 2° pyridine [lit. $[\alpha]_D^{25}$ −33.7° pyridine (3)].

Anal. Calcd. for $C_{43}H_{68}O_{10}$: C, 69.32; H, 9.20. Found: C, 69.35; H, 9.02.

β-Sitosteryl D-*Glucoside Tetrabenzoate*

β-Sitosteryl D-glucoside (100 mg.) was dissolved in 10 ml. of dry pyridine, and 1.0 ml. of benzoyl chloride was added. The mixture was heated at 100°C. for 1 hr. and then diluted with 50 ml. of distilled water. The precipitate that formed was extracted with one 50-ml. and two 25-ml. portions of diethyl ether. The ether extract was washed with a solution of 1% sodium carbonate and then with water before being concentrated to dryness. The residue was twice recrystallized from hot 95% ethanol, m.p. 190–190.3°C.; $[\alpha]_D^{20}$ +16.1 ± 2° chloroform [lit. m.p. 201°; $[\alpha]_D^{25}$ + 15.9° chloroform (3)].

Anal. Calcd. for $C_{63}H_{76}O_{10}$: C, 76.18; H, 7.71. Found: C, 76.13; H, 7.77.

Hydrolysis of β-Sitosteryl D-*Glucoside to β-Sitosterol*

β-Sitosteryl D-glucoside (500 mg.) was hydrolyzed by refluxing for 1½ hr. with a mixture consisting of 50 ml. of 4 *N* hydrochloric acid, 50 ml. of amyl alcohol, and 50 ml. of 95% ethanol. Most of the alcohols were removed by steam distillation, leaving the aqueous acid solution containing a white precipitate. The precipitate was collected on a filter, washed with water, and then twice recrystallized from hot 85% ethanol, m.p. 134–134.5°C.; $[\alpha]_D^{24}$ −37.1 ± 1.5° chloroform [lit., m.p. 137–138°C.; $[\alpha]_D^{20}$ −38.2° chloroform (3)].

Anal. Calcd. for $C_{29}H_{50}O$: C, 83.99; H, 12.15. Found: C, 83.63; H, 12.01.

Free β-sitosterol was also isolated from grapefruit seeds and from the acetone-soluble fraction obtained in the purification of β-sitosteryl D-glucoside. The sugar in the hydrolyzate was identified as glucose by methods previously described (4).

β-Sitosterol Acetate

This compound was prepared by the same method used to prepare β-sitosteryl D-glucoside tetracetate, m.p. 124–124.5°C.; $[\alpha]_D^{23}$ −40.2 ± 3° chloroform [lit., m.p. 125–126°C.; $[\alpha]_D^{22}$ −40.3° chloroform (3)].

Anal. Calcd. for $C_{31}H_{52}O_2$: C, 81.52; H, 11.48. Found: C, 81.45; H, 11.30.

β-Sitosterol Benzoate

This compound was prepared by the same method used to prepare β-sitosteryl D-glucoside tetrabenzoate, m.p. 144–144.5°C.; $[\alpha]_D^{23}$ −14.7 ± 3° chloroform [lit., m.p. 147–148°C.; $[\alpha]_D^{21}$ −13.6° chloroform (3)].

Anal. Calcd. for $C_{36}H_{54}O_2$: C, 83.34; H, 10.49. Found: C, 83.34; H, 10.37.

Infrared Data[5]

The β-sitosteryl D-glucoside was compared with a sample isolated from the juice of Valencia oranges by Swift (3). In the range 2–15 μ, the infrared curves were qualitatively identical in every detail. Because the

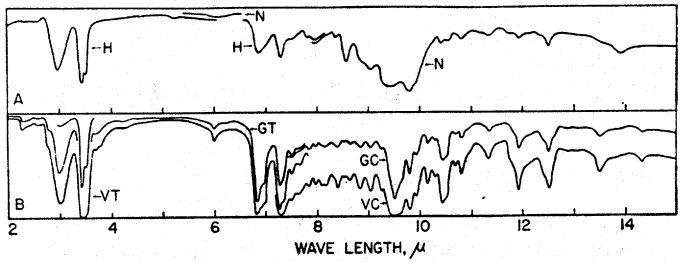

FIG. 1. Infrared absorption spectra. *A*, β-sitosteryl D-glucoside; *B*, β-sitosterol from grapefruit pulp (*G*) and from Valencia orange juice (*V*) (3). *H* and *N* represent mulls in hexachlorobutadiene and Nujol, respectively; *T* and *C* represent solutions in tetrachloroethylene and carbon disulfide, respectively, each in 0.13-mm. cells.

concentrations were nearly the same, both are adequately represented by a single curve, shown in Fig. 1. The mulling media were selected so as to avoid all foreign bands except the minor structures between 13.4 and 14 μ. Notable features are the broad, deep band from bonded hydroxyl groups at 2.97 and the general pattern of a broad, deep series of superimposed bands from 8.8 to 10 μ, frequently seen in polyoxygenated substances. Sample bands occurred at 2.97, 3.43, 3.50, 6.86, 7.30, 8.56, 8.85, 9.03, 9.4 (broad), 9.79, 10.41, 10.54, 10.80, 11.33, 11.74, 11.91, and 12.49 μ.

The β-sitosterol was compared with a sample from Swift (3). The

[5] We are indebted to Mr. J. S. Ard of this laboratory for the analytical results and infrared data. The latter were obtained with a Perkin-Elmer Model 12-C spectrometer, using a sodium chloride prism.

curves (Fig. 1) are qualitatively identical in every detail from 2 to 15 μ. The solvents chosen were found particularly satisfactory for sterols, and all the bands from the media were weak and were compensated for. Sample bands occurred at 2.28, 3.00 (bonded hydroxyl), 3.41, 3.50, 5.99 (Δ^5 ethylenic band), 6.84, 6.95, 7.26, 7.32, 7.50, 7.65, 7.84, 7.96, 8.09, 8.16, 8.38, 8.51, 8.62, 8.83, 9.03, 9.24, 9.50, 9.62, 9.79, 9.92, 10.14, 10.29, 10.43, 10.49, 10.68, 10.80, 11.34, 11.91, 12.50, 13.49, and 14.31 μ.

Antifungal Activity of β-Sitosteryl D-*Glucoside and β-Sitosterol*

These compounds in pure form inhibited the growth of *Fusarium oxysporum* f. *lycopersici*, W-R-5-6, and other fungi to some extent. Impure materials, however, gave larger inhibition zones. The difference in these results is attributed to an oily fraction, which in itself did not show antifungal activity but which apparently increased the solubility and rate of diffusion of the sterols.

Summary

β-Sitosteryl D-glucoside and β-sitosterol were isolated from commercially dried grapefruit pulp and from grapefruit seeds. Infrared data showed that these compounds are identical with those isolated from orange juice. These compounds were found to have a low order of antifungal activity.

References

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